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Redescription of the Sarcocysts of *Sarcocystis rileyi* (Apicomplexa: Sarcocystidae)

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ABSTRACT. The intermediate hosts for *Sarcocystis rileyi* (Stiles 1893) Minchin 1913 are ducks (*Anas* spp.), and the striped skunk (*Mephitis mephitis*) is its definitive host. The structure of sarcocysts from an experimentally infected shoveler duck (*Anas cylpeata*) fed sporocysts from an experimentally-infected *M. mephitis* was studied and compared with type specimens from a naturally infected duck. The experimentally infected duck was killed 154 d after feeding sporocysts. By light microscopy the sarcocyst wall was 3–5 μm thick with indistinct villar protrusions. Ultrastructurally, the sarcocyst wall was a type-23 cyst wall with anastomosing villar protrusions that were up to 7.5 μm long. The villar projections contained filamentous structures. The bradyzoites were 12–14 μm long. Structurally, the sarcocyst from the naturally infected and experimentally infected ducks appeared similar.

Key Words. Bradyzoites, sarcocyst wall, ultrastructure, villar protrusions.

INFECTIONS by the species of the genus *Sarcocystis* are considered common in many species of birds (Dubey, Speer, and Fayer 1989). Macroscopic sarcocysts in ducks are of particular interest because hunters often bring pieces of infected muscles for laboratory identification and these specimens have been used for teaching because of their easy availability. *Sarcocystis rileyi* (Stiles 1893) was placed originally in the genus *Balbianea*. It was named after Prof. C. V. Riley, who first reported the parasite in a duck (Riley 1869). Stiles (1893), in the same paper, described another species of *Sarcocystis*, *Balbiana falcatula* from muscles of the Rose-breasted Grossbeak (*Habia budoviciana*). These parasites were transferred to the genus *Sarcocystis* by Minchin (1903).

Life cycles of these parasites in birds remained unknown until 1978–1982 when the opossum *Didelphis virginiana* was found to be the definitive host for *Sarcocystis falcatula* (Box and Duszynski 1978; Box and Smith 1982; Duszynski and Box 1978), and the striped skunk (*Mephitis mephitis*) was shown to be the definitive host for *S. rileyi* (Cawthorn, Rainnie, and Wobeser 1981; Wicht 1981). Macroscopic sarcocysts have been reported from many species of ducks, water fowl, and geese from North America and all of these are considered to be *S. rileyi* (Beaudette 1941; Clark 1958; Cornwell 1963; Erikson 1940; Fedynich and Pense 1992; Gower 1938; Hoppe 1976; Riley 1931; Wobeser and Cawthorn 1982; Wobeser, Leighton, and Cawthorn 1981) although this species is rarely seen in Europe (Kalisinska et al. 2003). Ducks are also known to be intermediate hosts for several other species of *Sarcocystis* (Drouin and Mahrt 1980). At present, it is uncertain whether macroscopic sarcocysts in many species of ducks belong to one or more species of *Sarcocystis*. Stiles (1893) listed the shoveller or shovel-bill or spoon bill (*Anas clypeata*) and mallard or tame duck (*Anas boschus*) as hosts for *S. rileyi*. Among the specimens Stiles (1893) deposited in the United States National Parasite Collection (USNPC), Beltsville, Maryland, he marked those from the shoveller duck (*Anas clypeata*) as ''type'' and those from other hosts as either ''cotype'' (paratype) or ''voucher''. However, because he did not designate a holotype or select a type host in his paper, his type series must be regarded as syntypes. Following the recommendations of Articles 73 and 74 of the International Code of Zoological Nomenclature (ICZN) (1999) we designate a single slide of Stiles' specimens from sarcocysts in *Anas clypeata* as the lectohapantotype (see ICZN 1999). This will allow future workers to restrict the name *S. rileyi* to the species we redescribe herein from the shoveller duck should Stiles' type series be shown to include more than one species. The remaining specimens in Stiles' type series are designated paralectohapantotypes (see ICZN 1999). The additional specimens we used to redescribe *S. rileyi* have no name-bearing type status, but have been deposited in the USNPC (N.93077) as hypotypes (specimens used in a publication to redescribe a species (Frizzell 1933). Additional specimens of sarcocysts from *Anas boscus* and other ducks in USNPC should be called *Sarcocystis* spp. until a specific determination has been made.

Among the taxonomic criteria available at the present time, the structure of the sarcocyst appears to be the most reliable to determine *Sarcocystis* species within a given host (Dubey, Speer, and Fayer 1989). In the present study, we redescribe the structure of sarcocysts of *S. rileyi*, which until now has not been determined.

MATERIALS AND METHODS

Naturally infected ducks from Stiles collection. Stiles had deposited these specimens in 1893 after the publication of his paper. Therefore, there is no mention of these specimens in his paper. Serial sections of pectoral muscle of a duck (*Anas clypeata*) mounted on glass slides were obtained from the USNPC (no. 2). The sections were approximately 20 μm thick and stained with hematoxylin and eosin (H and E). For the present study these specimens were re-examined. In addition, serial sections from *A. boschus* were re-stained (USNPC No. 2476) and examined. Coverslips were removed from the original slides and restained with H and E because the stain in the original slides had faded. Stiles also deposited in USNPC breast muscle from a duck (*Anas clypeata*) containing macroscopic sarcocysts. These specimens had been preserved in 70% ethanol. For the present study, muscle from this duck (*A. clypeata*, from Stiles collection) were processed for light microscopy. These sarcocysts were in 70% ethanol were from the same duck. To avoid further confusion we are assuming that these specimens were from the same duck.

Experimentally infected duck. For the present study, sarcocysts were derived from a laboratory-raised shoveler duck (*Anas clypeata*), Duck No. 1. This duck was killed 154 d after feeding sporocysts from the feces of a skunk (Cawthorn, Rainnie, and Wobeser 1981). The skunk had been raised in the laboratory and sporocysts were collected from its feces after it had

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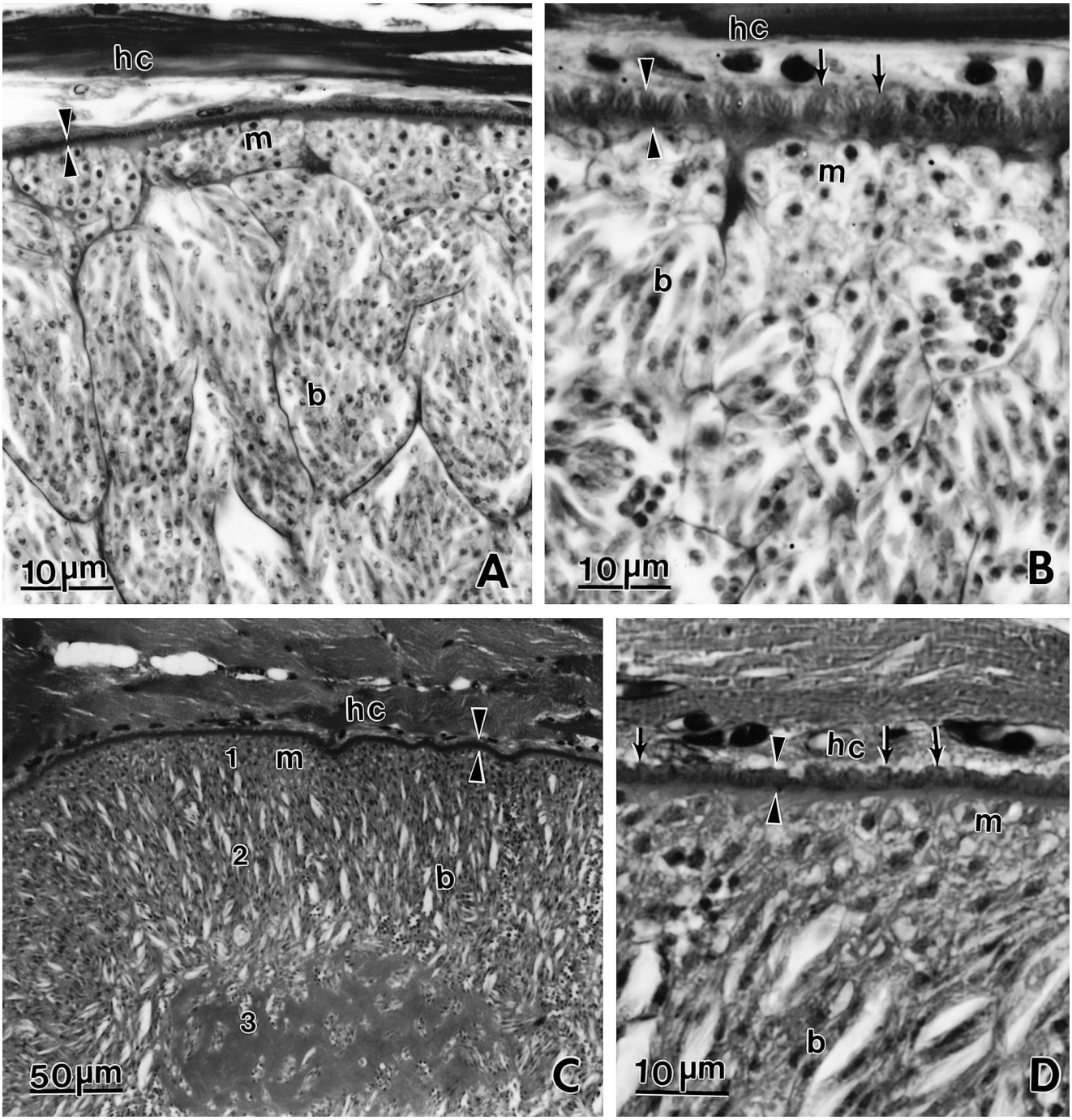

Fig. **1.** Structure of sarcocysts of *Sarcocystis rileyi* from a naturally-infected *Anas clypeata* from Stiles collection (**A, B**) and experimentally-infected *Anas clypeata* (**C, D**). Hematoxylin and eosin stain. Note host cells (hc), metrocytes (m), bradyzoites (b), and septa. The thickness of the sarcocyst wall is indicated by opposing arrowheads. **A.** The cyst wall appears smooth. **B.** Note slender villar protrusions (small arrows) on the sarcocyst wall. **C.** The cyst interior can be divided into three zones (1–3). Zone 1 contains metrocytes (m) and bradyzoites, zone 2 contains bradyzoites (b), and zone 3 has empty spaces or degenerated material. **D.** Note minute villar protrusions (small arrows) on the sarcocyst wall.

been fed muscles from naturally infected shoveler ducks (Cawthorn, Rainnie, and Wobeser 1981).

Light microscopic examination. For light microcopy, sections of muscles were fixed in 10% formalin, embedded in paraffin, sectioned at 5 µm, and examined after staining with H and E.

Transmission electron microscopic examination. For transmission electron microscopy, samples of muscle containing macroscopic cysts from Duck No. 1 were fixed in cold 2.5% (v/v) glutaraldehyde in Millonig's phosphate buffer (pH 7.2) at 4 °C. Tissues were post-fixed in 2% (w/v) osmium tetroxide, dehydrated in ethanol, and embedded in Epon. Ultrathin sections were stained with uranyl acetate and lead citrate, and examined with a JEOL 100 CX electron microscope.

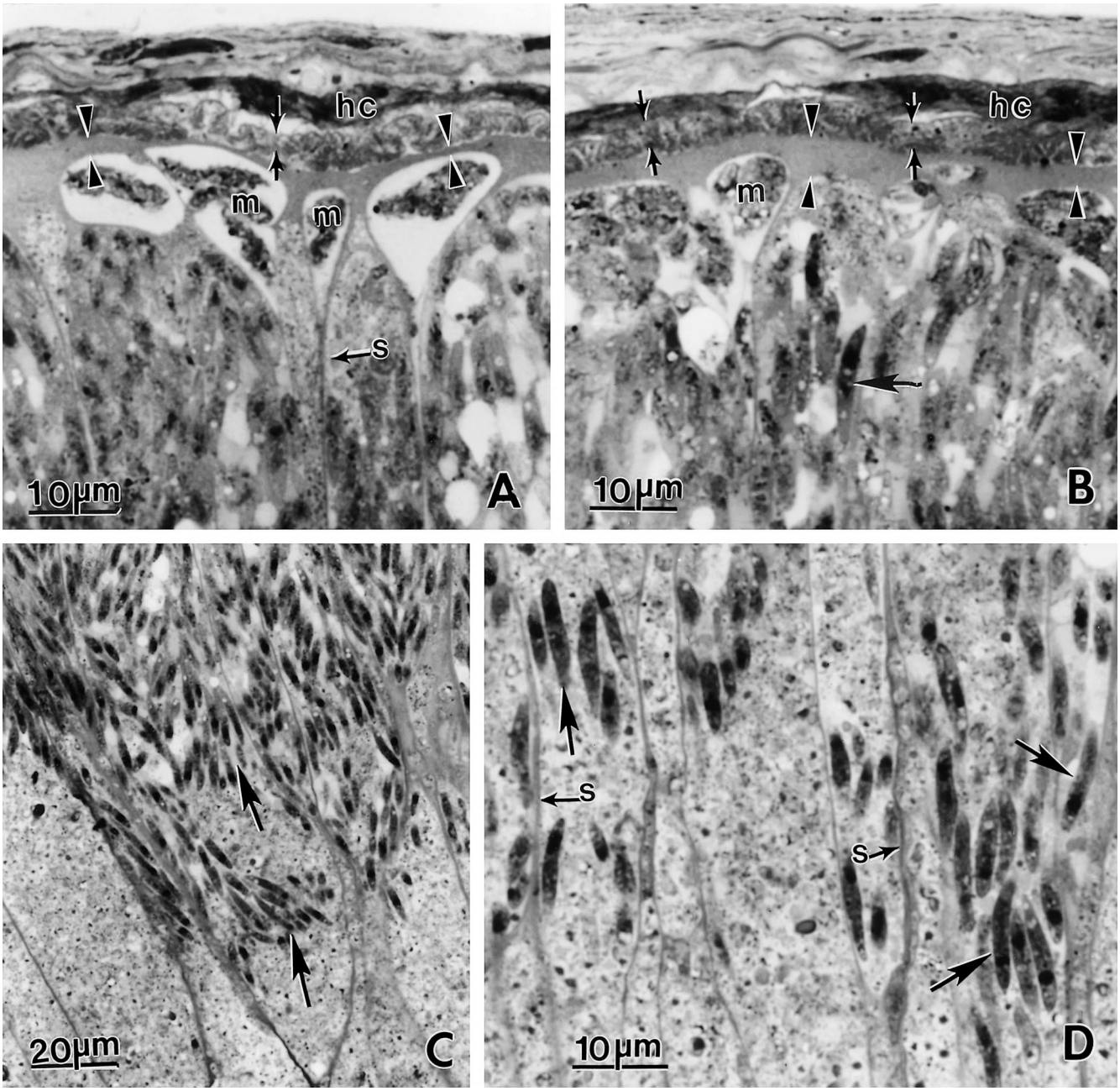

Fig. **2.** Structure of the sarcocyst of *Sarcocystis rileyi* from experimentally infected duck *Anas clypeata* in 1-µm section stained with Toluidine blue. The cyst wall has slender villar protrusions (opposing small arrows), and the homogenous granular layer of variable thickness (opposing arrowheads), continues into the sarcocyst interior as septa (s). Metrocytes (m) are located just below the cyst wall (**A, B**). Bradyzoites (bigger arrows) are arranged in groups, demarcated by septa (s) (**C, D**).

RESULTS

By light microscopy the structure of sarcocysts in the H and E-stained specimens of Stiles (1893) from *A. clypeata* and *A. boschus* was not clear. A few details of the sarcocyst wall were visible even in these thick sections). The cyst wall was approximately 3–5 µm thick without any visible villar protrusions. However, in H and E-stained sections obtained from Duck A (*A. clypeata*) details of sarcocyst wall were clear (Fig.1 A, B) and these resembled sarcocysts from Duck No. 1.

In the experimentally-infected duck, the sarcocyst could be divided into an outer zone containing the cyst wall, a middle zone containing bradyzoites, and the innermost zone containing degenerated material (Fig. 1C). The cyst wall appeared to be smooth and approximately 3 µm thick (Fig. 1D). Just beneath the cyst wall was the granular layer that continued into the interior of the cyst as septa. In 1-µm thick Toluidine blue-stained sections more details of the cyst wall became visible, including slender villar protrusions (Fig. 2). The granular layer was smooth and homogenous but of uneven thickness (Fig. 2A, B). Faintly stained metrocytes were located in the granular layer

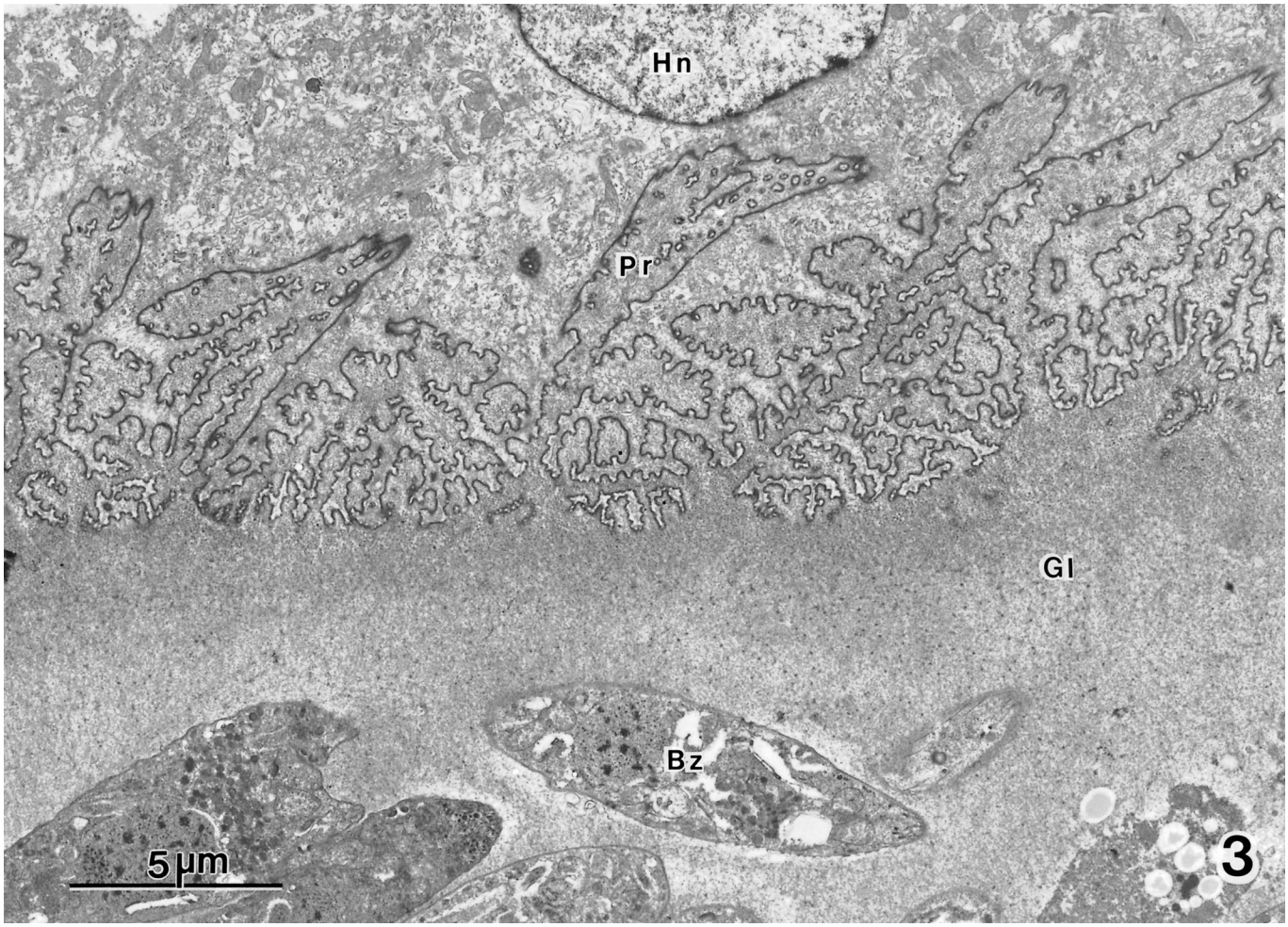

Fig. **3.** Transmission electron micrograph of a sarcocyst of *Sarcocystis rileyi* in the pectoralis major of Duck No. 1, *Anas clypeata*. The margin of the sarcocyst shows branched villar projections. Note the host-cell nucleus (Hn), granular layer (Gl), villar protrusions (Pr), and bradyzoites (Bz).

or just below it. The middle zone contained bradyzoites, which were arranged in rows and separated by septa. Bradyzoites were slender, and were approximately 11–13 μm long. The nucleus was located subterminally (Fig. 2C, D).

Ultrastructurally, the cyst wall was 68.2 nm thick (50.5–89.2 nm; n = 20) and consisted of an outermost parasitophorous vacuolar membrane (pvm) 9.3 nm thick (6.4–12.7 nm; n = 20) and an inner dense layer, which was 47.6 nm thick (33.3–58.4 nm; n = 20) (Fig. 3). The electron-dense layer of the cyst wall extended along the length of the sarcocyst wall (Fig. 3, 4), although it was absent at invaginations of the primary cyst wall (Fig. 5). The sarcocyst wall was folded into branched projections (Fig. 4). These projections were 7.5 μm long (2.6–9.5 μm; n = 20) and coalesced with each other. The villar projections contained filamentous structures, most of which were parallel to the long axis of the projections (86.6 nm long; 31.3–125 nm; n = 10) although some cross-sections were present (25.3 nm diam.; 12.8–41.7 nm; n = 10) (Fig. 6).

A moderately dense granular 2.9-μm (0.4–4.9 μm; n = 20) thick layer was present immediately beneath the villar projections (Fig. 4). Coarse granules (60.0 nm diam.; 41.7–75.0 nm; n = 10), most numerous in the outermost one-third of the granular layer, were present at the bases of the projections (Fig. 4). A few metrocytes (12.6 × 5.2 μm; 12.0–13.4 × 4.5–6.4 μm; n = 3) were at the periphery of sarcocysts; juxtapozed with the granular layer. A few metrocytes were also present in the interior of the sarcocyst among mature bradyzoites.

Numerous, tightly arranged bradyzoites were present below metrocytes. They were 12.2 × 3.4 μm (10.7–13.9 × 2.5–5.1 μm; n = 10). Single or groups of bradyzoites were separated by septa. The bradyzoites had double-membraned pellicle, contained a conoid, micronemes, rhoptries, amylopectin granules, dense granules, and a terminal nucleus (Fig. 7, 8). The small micronemes were numerous and were dispersed throughout the length of the bradyzoite, but were most abundant in the 1/3 of the conoidal end of the parasite (Fig. 7). The rhoptries had a long neck opening in the conoid (Fig. 7) and a bulbous end. No more than three rhoptries were seen in any one plane of section. Amylopectin granules were numerous and dispersed in four-fifths of the non-conoidal end.

DISCUSSION

Duszynski and Box (1978) reported that an opossum fed muscles from a naturally-infected pintail duck (*Anas acuta*) shed *Sarcocystis* sporocysts but five other opossums fed heavily-infected muscles from shoveller ducks did not shed sporocysts. Box and Duszynski (1978) reported that *Sarcocystis* sporocysts from opossums that were fed tissues of cowbirds and

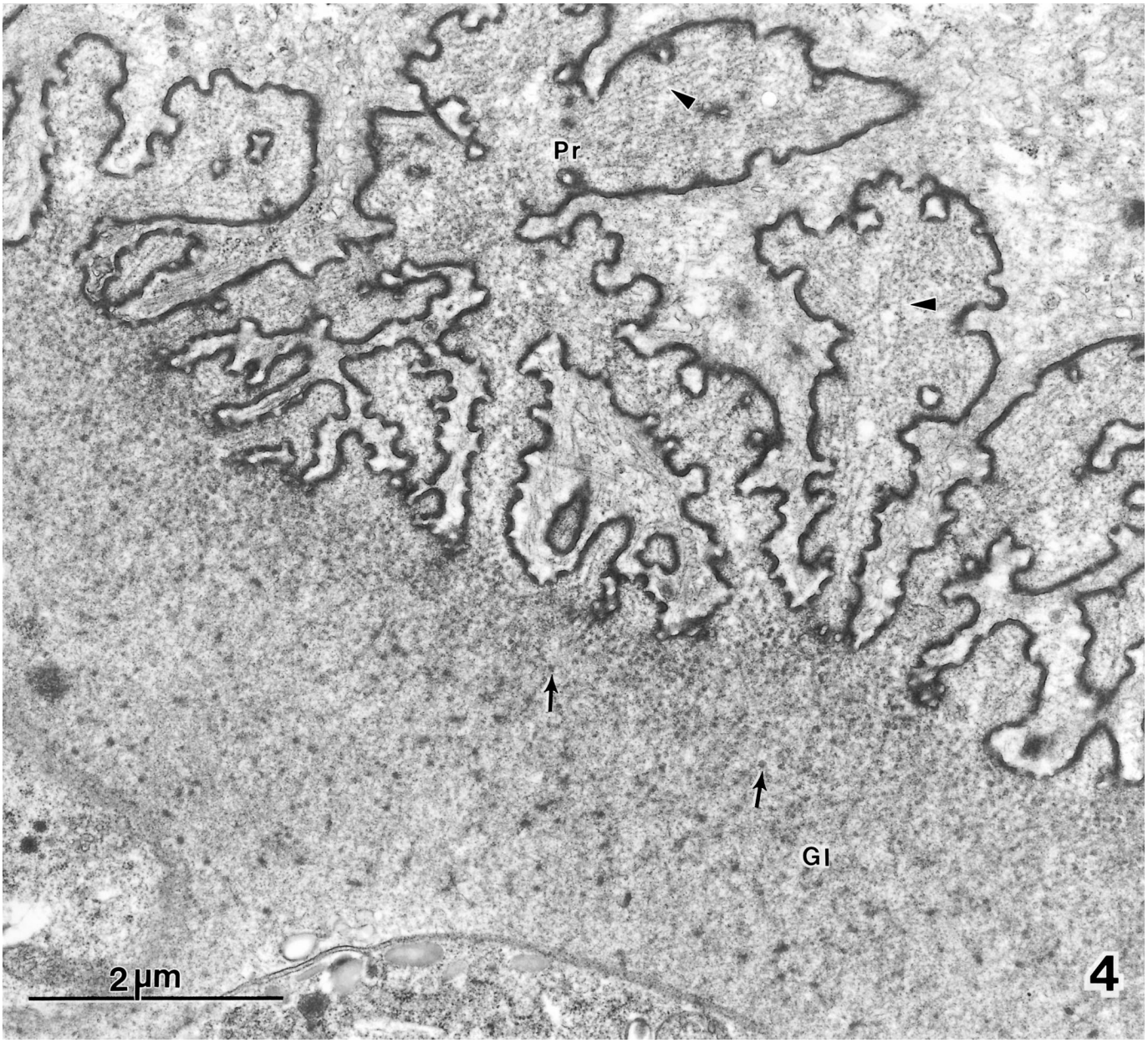

Fig. **4.** Higher magnification of section through margin of sarcocyst *Sarcocystis rileyi*, showing filamentous structures (arrowheads) in the villar protrusions (Pr) and granules (arrows) in the granular layer (Gl) at bases of protrusions.

grackles were not infective to laboratory-raised ducks (*Anas platyrhynchos*). These observations indicate that opossums are not a definitive host for *S. rileyi* of shoveller ducks and more than one species of *Sarcocystis* may form macroscopic sarcocysts in different species of ducks.

There is also the possibility that the opossum that shed the *Sarcocystis* sporocysts after ingesting pintail duck might have been naturally infected because it was not raised in the laboratory from pouch-derived opossums. Opossums are now known to be definitive hosts for at least four named (*Sarcocystis falcatula*, *Sarcocystis neurona*, *Sarcocystis lindsayi*, *Sarcocystis speeri*) and probably several unnamed *Sarcocystis* species (Dubey, Rosenthal, and Speer 2001; Dubey et al. 2001a, b; Spalding et al. 2002). For the present, the skunk is the only known definitive host for *S. rileyi*. Cats, dogs, coyotes, ferrets or mink are not definitive hosts for *S. rileyi* (Drouin and Mahrt 1979). Golubkov (1979) reported transmission of *Sarcocystis* species from ducks to cats in Russia but these findings have not been confirmed.

It is of interest that two groups of researchers independently reported shedding of *Sarcocystis* sporocysts in feces of skunks fed naturally-infected muscles from shoveller ducks from Colorado, USA (Wicht 1981), and Saskatchewan, Canada. The group in Canada completed the entire life and fulfilled Koch's postulates (Cawthorn, Rainnie, and Wobeser 1981).

Sarcocysts of *Sarcocystis* spp. are structurally distinct from all other cyst-forming coccidians because the cyst wall has villar protrusions and the interior is divided by septa. Dubey,

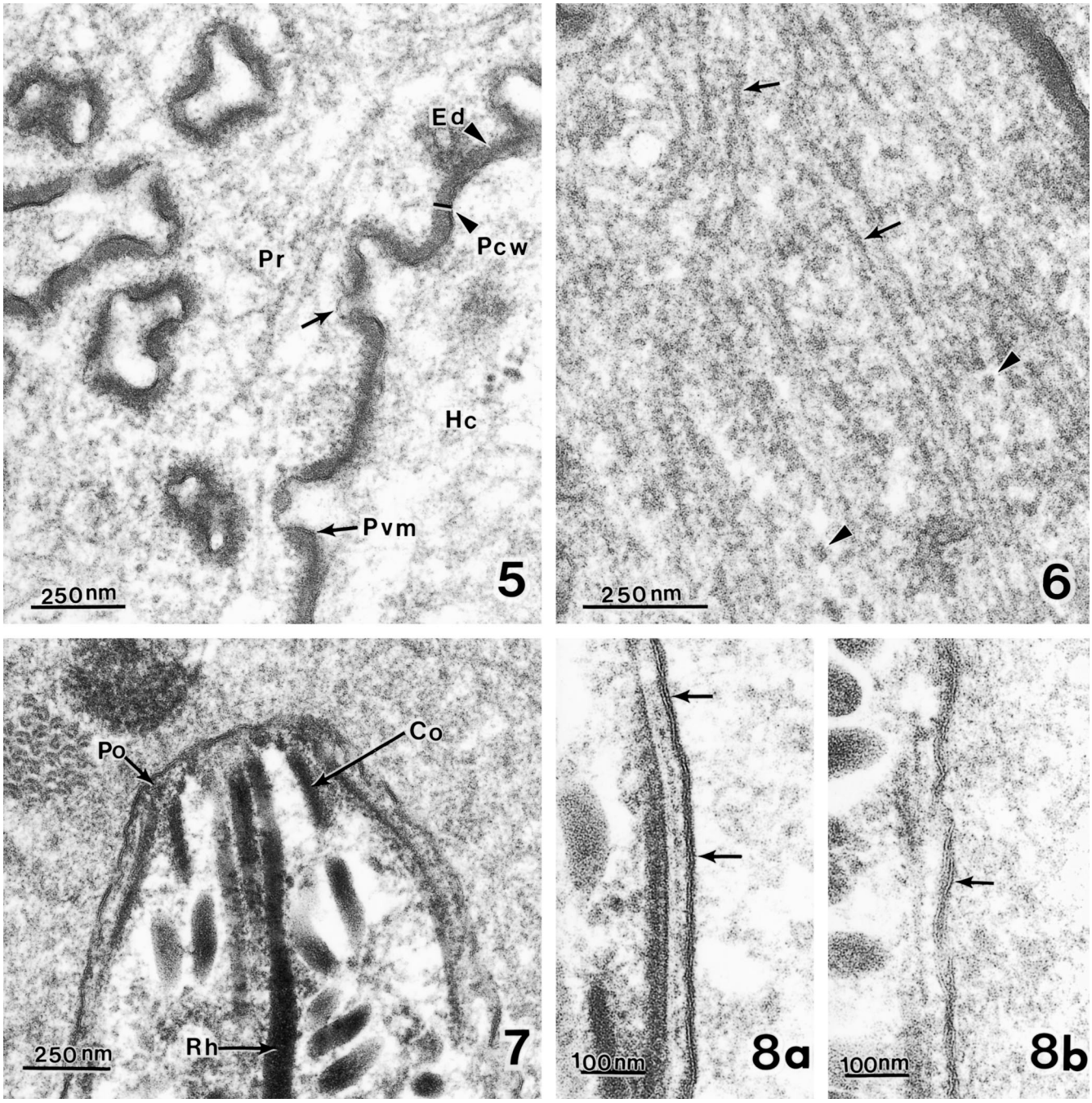

Fig. **5**–**8.** Sections of villar protrusions and bradyzoites of *Sarcocystis riley* from Duck No. 1. **5.** Detail of protrusion of the sarcocyst showing detail of cyst wall. Arrows indicate absence of a dense (Ed) layer at the invaginations of primary cyst wall (Pcw) beneath the parasitophorous vacuolar membrane (Pvm) adjacent to cytoplasm of host all (Hc). **6.** Filamentous structures (arrows) present in projection of cyst wall. Note presumed cross-sections of filamentous structures (arrowheads). **7.** Anterior region of bradyzoite. Note detail of apical complex: three rhoptries (Rh), conoid (Co), and polar ring (Po). **8a.** Double unit membrane (arrows) in pellicle of bradyzoite. Tight form. **8b.** Double unit membrane (arrow) in pellicle of bradyzoite. Loose form.

Speer, and Fayer (1989) and Dubey and Odening (2001) classified the villar protrusions into 37 structurally distinct types. The mature sarcocysts of *S. rileyi* have type-23 tissue cyst wall (Dubey, Speer, and Fayer 1989) characterized by anastomosing protrusions that contain fine granules and microfilaments. This type of sarcocyst wall has not been found in any other species of *Sarcocystis* (Dubey and Odening 2001; Dubey, Speer, and Fayer 1989).

In the same paper as he named *S. rileyi*, Stiles (1893) also named *S. falcatula* from the muscle of the Rose-breasted Grossbeak. *Sarcocystis falcatula*-like sarcocysts are structurally distinct from those of *S. rileyi* because the bradyzoites are half the size (i.e. 6 μm) of bradyzoites of *S. rileyi* (~ 12 μm) and they have type-9 sarcocyst wall with finger-like villar protrusions (Dubey, Speer, and Fayer 1989).

Because there is no definite way to confirm the species from

the original description of *S. rileyi* by Stiles (1893) the redescription of sarcocyst from experimentally infected *A. clypeata* should help identification of macroscopic sarcocysts in ducks.

Taxonomic summary.

Intermediate host: Shoveler duck (*Anas clypeata*) and probably other species of ducks.

Definitive host: Skunk (*Mephitis mephitis*)

Distribution: North America

Specimens (Hypotypes) deposited in USNPC: Histologic sections of sarcocysts in pectoral muscle from naturally infected Duck (*A. clypeata*) of Stiles (USNPC No. 93676); an experimentally infected Duck (*A.clypeata*) No. 1. Specimen No. 93677-1 (H and E stain), 93677-2 (Toluidine blue stain).

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